

**AMENDMENTS****IN THE SPECIFICATION:**

On the first page, after the title and before Background Of the Invention, please amend the paragraph presented in the Preliminary Amendment filed August 6, 2003 as follows:

This application is a Divisional of U.S. Serial No. 09/495,880 filed February 1, 2001, now Patent No. 6,667,150, which is a continuation of International Application PCT/EP98/04836, filed August 3, 1998, the contents of which are ~~said application~~ is incorporated herein in ~~its entirety~~ their entireties by reference hereto.

On page 16, please replace paragraph "c." with:

- c. The chloramphenicol resistance gene (CAT) assembled *via* assembly PCR (Ge and Rudolph, *BioTechniques* 22 (1997) 28-29) using the template pACYC (Cardoso and Schwarz, *J. Appl. Bacteriol.* 72 (1992) 289-293) is amplified by the polymerase chain reaction (PCR) with the primers:

CAT\_BspEI(for): 5' GAATGCTCATCCGGAGTTC **(SEQ ID NO 1)**

CAT\_Bsu36I(rev): 5' TTTCAGTGGCCTCAGGCTAGCACCGGCGTTTAAG **(SEQ ID NO 2)**

On page 16, please replace paragraph "a." with:

- a. The DNA encoding the C-terminal domain including the long linker separating it from the amino terminal domain of the filamentous phage pIII (gIII short) is amplified by PCR using pOK1 (Gramatikoff *et al.*, *Nucleic Acids Res.* 22 (1994) 5761-5762) as template with the primers:

gIII short(for): 5'GCTTCCGGAGAATTCAATGCTGGCGGCGGCTCT3' **(SEQ ID NO 16)**

gIII short(rev): 5'CCCCCACAAGCTTATCAAGACTCCTTATTACG3' **(SEQ ID NO 17)**

On page 17, please replace the paragraph designated "primers" with:

Primers: FR604 5' GTTCACGTAGTGGGCCATCG 3' (SEQ ID NO 43)  
 FR605 5' TGAGAGGTCTAAAAAGGCTATCAGG 3' (SEQ ID NO 44)  
 FR606 5' TAGCCTTTTGTAGACCTCTCAAAAATAG 3' (SEQ ID NO 45)  
 FR607 5' CGGTGTACAGACCAGGCGC 3' (SEQ ID NO 46)

Please replace paragraph 2.2 on pages 17-19 with:

## **2.2.: Proof of principle experiments**

Despite of the absence of the two originally associated IR mutations, the hybrid phage vector fjun\_1B-R408IR (carrying the chloramphenicol acetyltransferase conferring chloramphenicol resistance) could be co-transformed with a phagemid (pOK1deltajun, carrying the beta-lactamase gene conferring ampicillin resistance) containing a phage origin of replication. More importantly, fjun\_1B-R408IR could stably co-exist with the phagemid pOK1deltajun, and the phagemid was efficiently co-packaged together with the fjun\_1B-R408IR phage genome into polyphage particles. Titers of polyphages, simultaneously transducing chloramphenicol and ampicillin resistance, reached  $6 \times 10^8$  transducing units (t.u.)/ml of overnight bacterial culture K91 plating cells, a number almost equivalent to a titer of  $10^9$ /ml seen after selection on chloramphenicol only. Selection of the K91 transductants on ampicillin only gave a titer of  $5 \times 10^9$ /ml. These titers indicated that more than 50 % of all phages containing fjun\_1B-R408IR also contained the phagemid pOK1deltajun, thus representing polyphages. This high ratio of polyphages was confirmed by restriction analysis of transductants which had been selected on chloramphenicol only. More than 50 % of these clones also contained the phagemid in addition to the fjun\_1B-R408IR phage genome. fjun\_1B-R408IR was isolated in pure form from an individual transductant, which contained only this phage. The construct fjun\_1B-R408IR was used with pOK1deltajun for co-transformation of DH5 $\alpha$  cells, in order to produce selectively-infective phages (SIP) via fos-jun leucine zipper interaction (which non-covalently restores wt gIII function). Stable, double-resistant co-transformants were obtained with this combination and individual clones were grown overnight in the presence of cam/amp. The culture supernatant of these clones was filtered through a 45  $\mu$ M membrane filter and used to infect exponentially-growing F+ bacteria (K91 strain) for 20 min at 37 C. To test for the presence of infective SIP polyphages the cells were plated on LB agar plates

containing cam and amp and plates were incubated at 37 C overnight. Approx. 500 to 1000 transforming units (t.u.)/ml resulting in double-resistant transductants were obtained from individual co-transformants. DNA of those transductants was analyzed by restriction analysis which showed that 95 % (15/16 clones) of the clones had the correct pattern expected for fjun\_1B-R408IR and pOK1deltajun. Supernatants of several polyphage transductants were tested for persistent SIP phage production by re-infection of K91 cells. This confirmed that polyphage transductants continued to produce infective SIP phages and restriction analysis of the resulting 2<sup>nd</sup> round polyphage transductants showed that 44 % (14/32 clones) contained the correct vector combination. The rest of the clones contained the correct pOK1deltajun phagemid plus a recombined phage vector with a restored wt gIII, indicating an increase in recombination frequency when both vectors are propagated in the rec+ strain K91 (compared to the rec- strain DH5α used for co-transformation of IR phage and phagemid). To test other protein-protein interactions which give a higher titer of infective SIP phages and to verify the presence of hetero-polyphages (co-packaging of phage and phagemid instead of co-infection by monophages or homo-polyphages) , two peptide ligands (previously selected by SIP, WO97/32017) which bind to the p75 rat neurotrophin receptor (Chao et al., Science 232 (1986) 518-521) intracellular domain (p75ICD) were cloned as N-terminal gIIIc fusions in fjun\_1B-R408IR (replacing jun) and the phagemid pIG10.3, leading to constructs fpep3\_1B-IR3seq and pIG10.3-pep10 (WO97/32017), respectively, which contain the peptide pep3: 5'-TGTATTGTTTATCATGCTCATTATCTTGTTGCTAAGTGT-3' (SEQ ID NO 47) encoding the amino acid sequence (CysIleValTyrHisAlaHisTyrLeuValAlaLysCys (SEQ ID NO 48)) instead of the jun sequence. Sequencing of the respective parts of the transferred R408 fragment in fpep3\_1B-IR3seq revealed that neither of the two IR mutations (the G5986>A mutation from complementation group I in the gII 5' non-translated region, which should be found at position 3225 in fpep3\_1B-IR3seq, and the C143>T mutation (3789 in fpep3\_1B-IR3seq) from complementation group II leading to a Thr>Ile amino acid exchange in gII) were found to be present. However; the gII mutation G6090>T (3329 in fpep3\_1B-IR3seq), leading to a Leu>Val exchange, introduced by assembly PCR was present. Furthermore, three additional mutations compared to an f1 phage could be identified: G5737>A (2976 in fpep3\_1B-IR3seq) in the phage origin of replication, G343>A (3989) in gII, and G601>T (4247) in gII/X.

Please replace the second paragraph of page 20 with:

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